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(54) METHOD FOR DIAGNOSIS OF CANCER DISEASES.

(57) A method for diagnosis of cancer diseases, which comprises measuring the amount of UDP-N-acetyl-glucosamine: glycoprotein N-acetylglucosaminyl-transferase in the body fluid and diagnosing liver diseases based on an increase in said amount. Conventional diagnosis of liver cancer has been conducted by using as the tumor marker AFP, CEA or γ-glutamyltranspeptidase. Since, however, the positiveness ratios of these markers are about 60 %, early-stage detection has been nearly impossible. This method enables perfect early-stage detection of liver cancer by using UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyl-transferase as the tumor marker.

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Diagnostic Method of Cancerous Diseases

Field of the Invention

This invention relates to a method of diagnosing cancerous diseases.

More particularly, it relates to a method of diagnosing cancerous diseases of the liver, etc. based on the increase in the amount of UDP-N-acetyl-glucosamine:glycoprotein N-acetylglucosaminyltransferase (hereinafter abbreviated as Gn-T-III) in body fluid.

The method of this invention allows simple diagnosis of cancerous diseases such as hepatic cancer (hepatocirrhosis) by measuring the increase in the amount of Gn-T-III in body fluid (e.g., serum, saliva and urine), and hence will be of much benefit to the medical and diagnostic fields.

Prior Art and Problems to be Solved by the Invention

GOT, GPT, LDH, ChE and many other test items have been adopted for general diagnosis of hepatic functions.

These test items, however, are no more than to check the comparative degree of hepatic functions, and are far from direct diagnosis of hepatic diseases, particularly hepatic cancer.

Measurement of tumor markers, such as AFP and CEA, is also known to be necessary for the diagnosis of hepatic cancer and has been put into practice.

But these conventional tumor markers show a positivity rate of 60 % at the highest, making early diagnosis almost impossible.

Recently, γ-glutamyltranspeptidase is receiving attention as a new tumor marker (particularly for hepatic cancer), because of the new fact that the blood of patients with hepatic cancer contains glycoproteins carrying different sugar-chain structure compared with normal subjects. However, this γ-glutamyltranspeptidase is not better than AFP, CEA and others as tumor marker.

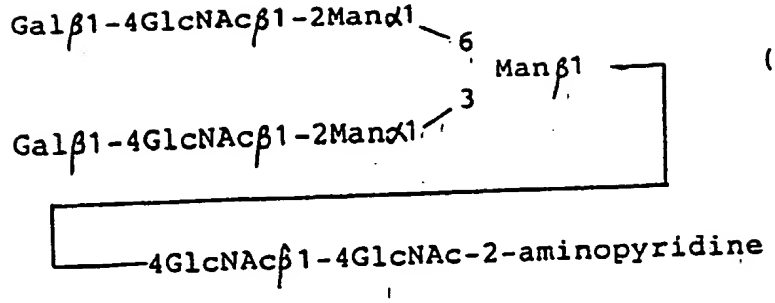
Means to Solve the Problems

Detailed studies on the change in sugar-chain structure in patients with hepatic cancer revealed that N-acetylglucosamine has attached, through β-1,4-linkage, to the mannose (of β-1,4-linkage) bound to the trimannosyl core of sugar chain of asparagine linked type. We continued our investigation on the assumption that this change might be accompanied by the increase in the amount of Gn-T-III — an enzyme capable of transferring this N-acetylglucosamine. As a result, it was demonstrated that the sera of patients suffering hepatic diseases (particularly hepatic cancer) show a significantly higher Gn-T-III activity compared with normal subjects. We then succeeded in establishing a simple method for measuring the amount of this enzyme. The present invention was accomplished on the basis of these findings.

It was first found by the present inventors that the sera of normal subjects generally show a Gn-T-III activity as low as about 2.0±0.5 nmol/ml/h, while the sera of patients with hepatic cancer about 2 to 3 times the activity, the sera of patients with hepatocirrhosis about 1.5 times and the sera of patients with chronic hepatitis 1.2 times.

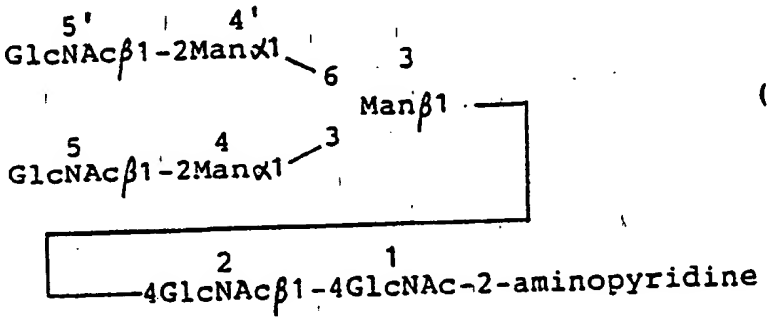
On page 634 of Preliminary Notes for the 60th Meeting of Japanese Biochemical Society, is described a method of measuring Gn-T-III activity, in which N-acetylglucosamine is transferred to GnGn sugar chain and the product thus formed is measured by high-performance liquid chromatography. However, it is not known at all to apply this method to the diagnosis of cancerous diseases.

In the method of this invention, the amount of Gn-T-III is preferably measured by allowing it to act upon uridine diphospho N-acetylglucosamine (hereinafter abbreviated as UDP-GlcNAc) and to transfer N-acetylglucosamine to GnGn sugar chain. Thus the product formed is detected by high-performance liquid chromatography. In this case, if the GnGn sugar chain is previously fluorescence-labelled, the product can be easily detected by monitoring the fluorescence intensity. The GnGn sugar chain used in this invention is isolated from human transferrin, and then pyridylaminated (fluorescence labelling) by the method of Hase et al. (.S. Hase et al, Journal of Biochemistry, 197-203 (1984)), as shown by formula (I).



(I)

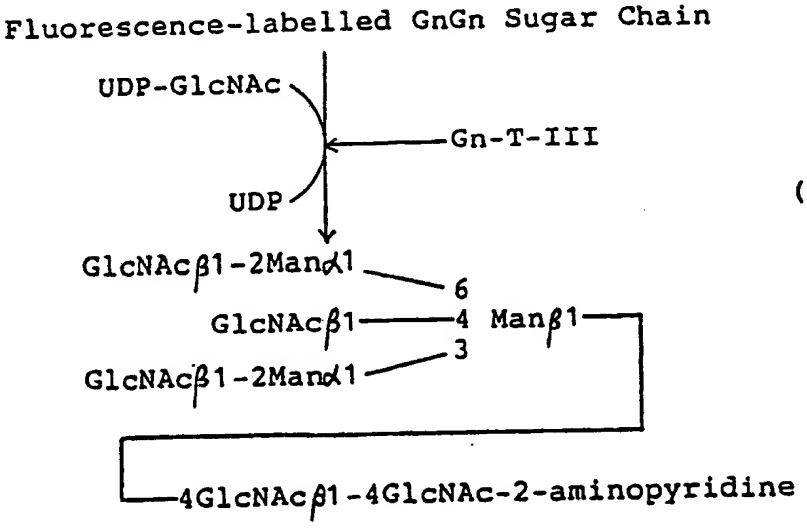
β-Galactosidase is then allowed to act upon this sugar chain, giving pyridylaminated GnGn sugar chain of formula (II).



(II)

The GnGn sugar chain herein means the part of compound (II) from which 2-aminopyridine (fluorescent substance) is removed, and it also includes a derivative thereof in which fucose is attached to the 1-position (GlcNAc).

The reaction of Gn-T-III in the method of this invention is shown by the following equation (III):



(III)

The reaction mixture was subjected to high-performance liquid chromatography, and the amount of reaction product was determined from the fluorescence-intensity, thus measuring the enzyme activity of Gn-T-III.

The amount of Gn-T-III may also be measured by other methods, such as by the antigen-antibody reaction.

Effects Achieved by the Invention

It was demonstrated that hepatic disease increases the Gn-T-III activity in the serum, and that this enzyme activity can be easily measured by allowing it to act upon UDP-GlcNAc to transfer N-acetylglucosamine to GnGn sugar chain and determining the amount of reaction product by high-performance liquid chromatography. This invention provides a simple method for diagnosing cancerous diseases such as hepatic cancer based on these findings.

Presented below is an Example of this invention.

Example

Reagent	
250 mM	MES (2-(N-morpholino)ethanesulfonic acid monohydrate) (pH: 6.25)
400mM	GlcNAc (N-Acetylglucosamine)
20mM	$MnCl_2$
40mM	UDP-GlcNAc
1.0%	Triton X-100
150μM	GnGn sugar chain (flurorescence-labelled)

Into fifty containers each containing 50 μl of the above reagent, were added 50 μl of sera taken from patients with primary hepatic cancer, patients with hepatocirrhosis, patients with chronic hepatitis, patients with fatty liver and normal persons (10 cases each), the mixtures were incubated at 37°C for one hour, and the reaction was terminated by adding 20 μl each of a solution containing 0.2M EDTA and 0.1M sodium borate.

Each of the reaction mixtures (1 μl) was subjected to high-performance liquid chromatography, fluorescene-intensity chromatograms were prepared, and the Gn-T-III relative activity was determined for each case.

The result is shown in Table 1 below.

Table 1

	Gn-T-III Relative Activity
Serum of patients with primary hepatic cancer	3.7±2.3
Serum of patients with hepatocirrhosis	3.3±1.8
Serum of patients with chronic hepatitis	2.0±0.5
Serum of patients with fatty liver	2.0±0.5
Serum of normal persons	2.0±0.5

Claims

1. A method of diagnosing cancerous diseases which comprises measuring an amount of UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase in body fluid and diagnosing hepatic diseases based on an increase in said amount.

2. The method for diagnosing cancerous diseases as defined in claim 1, wherein the amount of UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase is measured by allowing it to act upon uridine diphospho N-acetylglucosamine to transfer N-acetylglucosamine to GnGn sugar chain and determining an amount of reaction product thus formed.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP88/00898

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl[4] C12Q1/48

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C12Q1/48

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Nihon Nogei Kagakukai-hen [Nihon Nogei Kagakukai ABC Series (4) Koso-Biotechnology eno Shishin-I] 20 March 1985 (20. 03. 85) Asakura Shoten (Tokyo) P.94-114	1, 2
A	Seikagaku (The Sixtieth times, Nippon Seikagakukai Taikai Shoroku-Go) Vol. 59, No. 8, August. 1987 (Tokyo) Fujii Shigeru, Nishikawa Atsushi, Taniguchi Naoyuki ['H-NMR niyoru Ushi no IgG no Tosa Kozo no Kaiseki] P.634	1,.2

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
November 11, 1988 (11. 11. 88)	November 28, 1988 (28. 11. 88)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	